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Clinical evaluation of lymphocyte sub-populations and oxygen radical production in sarcoidosis and idiopathic pulmonary fibrosis

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The purpose of this study was to investigate the relationship between bronchoalveolar lavage (BAL)-derived parameters of interstitial lung disease and clinical and lung function parameters in 34 patients with sarcoidosis and 23 patients with idiopathic pulmonary fibrosis (IPF). BAL findings of healthy individuals served as controls.

Cell content and differentiation of BAL fluid were determined. Oxygen radical (O_2^-) production of BAL cells and of blood polymorphonuclear (PMN) cells was measured. Phenotypes of lung and blood lymphocytes were determined by immunoperoxidase staining. In addition, lung function was assessed, chest X-rays were made and serum ACE was measured.

Lymphocyte alveolitis in sarcoidosis was associated with increased alveolar macrophage (AM) O_2^- production ($P<0{\cdot}025$ *vs.* sarcoidosis with normal lymphocyte counts). Patients with extrapulmonary sarcoidosis had higher CD4/CD8 ratios in BAL ($P<0{\cdot}025$) and shorter disease duration ($P<0{\cdot}01$) than those with strictly pulmonary sarcoidosis. Disease duration in sarcoidosis correlated inversely with the number of BAL cells ($r=-0{\cdot}38$, $P<0{\cdot}05$), the relative and absolute number of lymphocytes in BAL fluid ($r=-0{\cdot}34$, $P<0{\cdot}05$ and $r=-0{\cdot}44$, $P<0{\cdot}01$, respectively) and the percentage of CD4-positive cells and the CD4/CD8 ratio ($r=-0{\cdot}43$, $P<0{\cdot}05$ and $r=-0{\cdot}48$, $P<0{\cdot}025$, respectively). Although significant increases in O_2^- production by BAL cells were observed in both IPF and sarcoidosis, only in sarcoidosis was a higher AM O_2^- production associated with a significantly lower total lung capacity ($r=-0{\cdot}67$, $P<0{\cdot}005$) and pulmonary diffusing capacity T_LCO ($r=-0{\cdot}50$, $P<0{\cdot}05$).

In conclusion, our findings show that lung lymphocyte phenotypes differ among patients with pulmonary and extrapulmonary sarcoidosis and that O_2^- production is upregulated in active sarcoidosis. In addition, our findings suggest that different relationships between BAL data and lung function in patients with sarcoidosis and IPF may be explained by differences in disease duration. In IPF, disease duration is likely to be underestimated because of its insidious onset. In sarcoidosis, the presence of extrapulmonary symptoms, helpful to establish an early diagnosis, is associated with significant BAL lymphocytosis.

Introduction

Sarcoidosis and idiopathic pulmonary fibrosis (IPF) are two disease entities belonging to the group of illnesses called interstitial lung diseases (ILD), which are characterized by distortion of lung parenchyma architecture. Two major problems present themselves in the clinical evaluation and management of these diseases. First, lung tissue, i.e. lung biopsy, is often required to establish a correct diagnosis. Secondly, an assessment of disease activity is necessary for treatment follow-up.

Bronchoalveolar lavage (BAL) provides a reliable, easy and safe way to assess inflammatory changes in the lungs (1–3) and has been used to identify cellular and soluble parameters of disease activity in patients with ILD. Increased numbers of polymorphonuclear cells (PMN), i.e. neutrophils and eosinophils, as well as lymphocytes and alveolar macrophages (AM) may be present in BAL fluid of patients with ILD (1,2,4). These alveolar inflammatory changes have been designated as 'alveolitis'. Depending on the dominant

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cell type, lymphocyte alveolitis, as usually found in sarcoidosis, and neutrophil alveolitis, as commonly found in IPF, can be distinguished. Soluble parameters of disease activity and cell activation include reactive oxygen metabolites, e.g. O_2^- (5–7) and lymphokines. In patients with ILD, increased O_2^- production has been demonstrated (5,8) and implicated in the pathogenesis of ILD. Lymphocytes may play an important role, both by regulating the release of O_2^- by AM and through the release of several lymphokines (9–12). In addition, studies have shown that in sarcoidosis, the ratio of CD4-positive or inducer lymphocytes to CD8-positive or cytotoxic lymphocytes (CD4/CD8 ratio) in the lung is generally increased (13–15), whereas in IPF this ratio is more likely to be decreased (13).

In the present study we investigated the relationship between the afore-mentioned BAL-parameters and conventional measures of extent and activity of disease, including clinical symptoms, pulmonary function, chest X-ray and serum angiotensin converting enzyme. Patients with sarcoidosis and patients with IPF were studied with respect to these parameters. The results of both patient groups were compared with each other.

Methods

STUDY SUBJECTS

Twenty-three patients with idiopathic pulmonary fibrosis (IPF) and 34 patients with newly diagnosed biopsy-proven sarcoidosis were included in the study. From our laboratory database data from 24 healthy individuals were available for comparison.

BRONCHOALVEOLAR LAVAGE

Bronchoalveolar lavage (BAL) was performed with a flexible bronchoscope after local anaesthesia of the airways with lidocaine spray. After rinsing the suction canal with 0·3% gelatin solution, the tip of a flexible bronchoscope (Olympus B1-IT20, Olympus Optical Co., Tokyo, Japan) was wedged in the lateral segment of the right middle lobe. Subsequently, ten aliquots of 20 ml of phosphate buffered saline of 37°C were instilled and recovered by gentle vacuum suction after each aliquot.

ISOLATION OF POLYMORPHONUCLEAR CELLS FROM BLOOD

Fifty millilitres of heparinized venous blood was diluted 1:1 with saline solution for isolation of PMN as described previously (16). In short, the red cell pellet obtained after Ficoll–Paque separation was processed by Dextran sedimentation. The mononuclear cell fraction was removed and processed separately for isolation of lymphocytes (see below). The dextran plasma layer containing PMN and some erythrocytes was collected and centrifuged. The cell pellet was resuspended in PBS and was gently layered on a Percoll solution and centrifuged for 20 min, after which the PMN layer was removed and diluted with PBS to be centrifuged and subsequently washed with PBS and finally resuspended in Hank's Balanced Salt Solution (HBSS+0·1% glucose). The volume and cell content of the suspension was determined and served to prepare cytocentrifuge preparations.

ISOLATION OF LYMPHOCYTES FROM BLOOD

The mononuclear cell fraction obtained after Ficoll–Paque separation of peripheral blood was washed twice during 10 min at 4°C at 550 *g* and 250 *g* (1800 and 1200 rpm, respectively) and resuspended in HBSS with a maximum volume of 4 ml. The number of cells in the suspension was determined in a Coulter counter. Cytospin preparations were made, fixed with acetone and stored at −80°C for subsequent immunological analysis.

WORK-UP BAL CELLS

The lavage fluid was filtered through a venous infusion filter (Curapharm®, Medica B.V. Hospital Supplies, The Netherlands) to remove mucus and centrifuged at 550 *g* (1800 rpm) at 4°C for 10 min. The unconcentrated supernatant was stored at −80°C for subsequent analysis. The cell pellet was resuspended and washed twice with centrifugation at 4°C for 10 min at 675 *g* (2000 rpm) and 330 *g* (1400 rpm), respectively. Cytospin preparations were made from 100-μl aliquots of the lavage cell suspension ($0{\cdot}3 \times 10^6$ cells ml^{-1}) on BSA-coated slides. Two preparations from each patient were stained with May-Grünwald/Giemsa. Cell differential counts of 500 cells were performed on two slides and the average count of both slides was taken as the definite cell differential. Additional acetone fixed cytospins were stored at −80°C for future lymphocyte staining.

OXYGEN RADICAL PRODUCTION

Oxygen radical production of BAL cells and blood PMN was measured as the superoxide dismutase (SOD) inhibitable reduction of cytochrome *c* as described previously (16). In short, a volume of cell suspension containing 4×10^5 cells was incubated with a mixture containing cytochrome *c* with or without SOD and Phorbol Myristate Acetate (PMA, Sigma) as a soluble stimulus at concentrations of 5, 10 and 20 ng ml^{-1}. The incubation

mixture, at a final volume of 0·5 ml, was collected in polystyrene tubes. Tubes without PMA were included to measure spontaneous reduction, and the total reduction was checked by including tubes with 0·085 ml of a saturated sodium hydrosulfite solution (Sigma). The reaction mixuters were prepared at room temperature and placed in a water bath at 37°C for 10 min. After termination of the reaction by centrifugation the optical density at 550 nm wave length was determined in a spectrophotometer. The oxygen radical production was expressed as nmol cytochrome *c* reduction per 15 min per 400 000 cells.

For the BAL cells, oxygen radical production was corrected for the percentage of lymphocytes, since lymphocytes are not capable of producing O_2^-. Consequently, O_2^- production was expressed as nmol cytochrome *c* reduction per 15 min per 400 000 O_2^- producing cells, i.e. AM, eosinophils and neutrophils (17). In healthy controls and patients with sarcoidosis, who all had less than 10% of eosinophils and neutrophils, the proportion of these cells in the total O_2^- production was considered neglectable and O_2^- production was again adjusted to be expressed as nmol cytochrome *c* reduction per 15 min per 400 000 AM.

LYMPHOCYTE SUBSET STAINING

Lymphocyte phenotypes of cytospin preparations of BAL cells and blood lymphocytes were determined by the immune peroxidase method using monoclonal antibodies against CD3, CD4, CD8, CD22 and HLA-Dr (Becton Dickinson). Cytospin preparations were washed in PBS of pH 7·2 for 5 min before and after a 45 min incubation with the respective monoclonal antibodies. Subsequently the cytospins were incubated with peroxidase-labelled rabbit anti-mouse-Ig serum (Dako Copenhagen) for 45 min followed by another washing in PBS for 5 min. The substrate for the enzymatic reaction, 3-amino, 9-ethylcarbazol was added. After 10 min the preparations were washed with a citrate buffer and rinsed with distilled water. Preparations were stained with Mayer's haematoxilin, rinsed briefly in water and mounted in Kaiser's glycerin-gelatin. At least 300 lymphocytes were counted and the numbers of cells positive for the respective antibodies were expressed as a percentage of the total number of lymphocytes.

LUNG FUNCTION

Lung volumes were measured by helium dilution according to standardized techniques. Predicted values according to the EGKS (18) were applied. Other spirometric parameters such as slow inspiratory vital capacity (IVC), forced expiratory volume in one second (FEV_1), inspiratory volume in one second (FIV_1) were measured with a standard water-sealed spirometer. FEV_1/IVC and FIV_1/IVC ratios were calculated to correct for restriction. Values of IVC, FEV_1, FIV_1 and total lung capacity (TLC) were expressed in litres BTPS (body temperature, pressure, saturated). The transfer factor for carbon monoxide (*T*LCO) was determined using the single breath technique of Krogh, as modified by Ogilvie (19) and Cotes (20). The components of *T*LCO, the diffusing capacity of the alveolocapillary membrane (D_m), and the pulmonary capillary blood volume (V_c), were determined from triplicate measurements of *T*LCO at high (88%) and low (19·2%) inspiratory oxygen concentrations. The *T*LCO values, breathing air, were corrected for haemoglobin concentrations according to Cotes (20) to obtain *T*LCO values under standard conditions. D_m and V_c were calculated following the equation originally devised by Roughton and Forster (21). *T*LCO and D_m were expressed in mmol kPa^{-1} min^{-1}, V_c was expressed in ml. Predicted values were taken from Cotes (20). All pulmonary function tests were performed at room temperature, with the patient in a steady state condition. Pulmonary function parameters were considered abnormal when below 80% of the predicted value.

ADDITIONAL STUDIES

Chest X-rays were performed according to routine procedures, judged for the presence of interstitial changes, and graded accordingly to well accepted criteria.

Determination of serum angiotensin converting enzyme (ACE) was performed as part of the patient characterization.

DATA ANALYSIS

Data were analysed with the SPSS/PC+statistical package, version 3·0. For between group comparisons Student's *t*-test or the Mann–Whitney ranked sum test were used. For comparison of multiple groups one-way analysis of variance followed by Duncan's multiple range test was used. Correlations were computed with Spearman's ranked correlation test. Logarithmic transformations were used when necessary to normalize distributions of test variables. The null hypothesis was rejected when it had a probability (*P* value) of 5% or less. Individual *P*

Table 1 Demographic and BAL characteristics of the subjects (mean ± SEM)

		Healthy	IPF	Sarcoidosis
Number		24	23	34
Age		41·7 ± 2·7	58·2 ± 2·9**	35·1 ± 1·7††
Sex (male/female)		17/7	12/11	23/11
Smokers		6	5	6
Disease duration (years)		—	2·04 ± 0·66	1·59 ± 0·51
Treated		—	3	7
Recovery	(%)	72·2 ± 2·9	64·9 ± 3·0	63·6 ± 2·8
Cells ml^{-1}	($\times 10^3$)	163·5 ± 35·1	321·2 ± 56·8**	221·6 ± 25·4**
Alv. mac.	(%)	89·7 ± 1·2	61·5 ± 6·4**	72·4 ± 3·2***
	($\times 10^3$ ml^{-1})	149·6 ± 33·6	196·3 ± 48·6	156·6 ± 20·0
Neutrophils	(%)	1·3 ± 0·3	13·9 ± 4·5***	1·8 ± 0·3†††
	($\times 10^3$ ml^{-1})	2·29 ± 0·76	54·8 ± 34·6***	4·18 ± 1·19†††
Eosinophils	(%)	0·29 ± 0·13	7·8 ± 2·7***	0·73 ± 0·17*††
	($\times 10^3$ ml^{-1})	0·72 ± 0·47	13·6 ± 4·2***	1·77 ± 0·51*††
Lymphocytes	(%)	8·6 ± 1·0	16·4 ± 4·1	25·1 ± 3·3***†
	($\times 10^3$ ml^{-1})	10·8 ± 1·7	55·9 ± 18·2*	59·0 ± 11·0***
		n=8	n=8	n=25
T-cells	(%)	71·5 ± 2·05	91·8 ± 1·91**	89·1 ± 1·67***
	($\times 10^3$ ml^{-1})	5·25 ± 1·16	70·0 ± 23·7**	58·9 ± 11·0***
CD4+ cells	(%)	45·0 ± 2·3	34·9 ± 9·4†	56·7 ± 3·4*
	($\times 10^3$ ml^{-1})	3·29 ± 0·73	24·9 ± 9·7*	40·6 ± 8·6***
CD8+ cells	(%)	32·3 ± 1·7	49·8 ± 9·3†	24·0 ± 2·7*
	($\times 10^3$ ml^{-1})	2·35 ± 0·53	40·8 ± 16·6**	15·2 ± 2·44**
CD4+/CD8+ ratio		1·40 ± 0·02	2·81 ± 2·1†	4·13 ± 1·0
B-cells	(%)	11·3 ± 0·7	2·38 ± 0·50**	1·60 ± 0·28***
	($\times 10^3$ ml^{-1})	0·84 ± 0·19	2·03 ± 0·85	1·20 ± 0·38
HLA-Dr+	(%)	1·25 ± 0·37	24·4 ± 8·9**	13·5 ± 3·1***
	($\times 10^3$ ml^{-1})	0·063 ± 0·021	24·0 ± 16·2**	7·14 ± 1·42***

Differences with healthy controls: *: $P<0.05$; **: $P<0.005$; ***: $P<0.0005$.
Differences between IPF and sarcoidosis: †: $P<0.05$; ††: $P<0.005$; †††: $P<0.0005$.

values are indicated in parentheses in text and by symbols in figures.

Results

SUBJECT CHARACTERISTICS

Table 1 presents the clinical and BAL data of the subject groups. Patients receiving treatment, either corticosteroids or immunosuppressive drugs, were not significantly different from untreated patients with respect to BAL parameters, lung function and lymphocyte subsets. Therefore, they were all included in all analyses.

Demographic characteristics

Patients with IPF were significantly older than the other two groups ($P<0.005$ in both analyses). There were significantly more patients with a known duration of complaints and/or symptoms of less than 3 months in the group of patients with sarcoidosis than in the group of patients with IPF ($P<0.05$).

BAL cell differentiation

The total cell count per ml BAL fluid was increased in both patient groups as compared to normals. Because of these differences the absolute numbers of the various cell types are included in the table. Based on the BAL cell differentiation in our healthy controls, BAL cell differentiations in patients were considered to be normal when the lymphocyte percentage was ≤ 17 and the combined neutrophil and eosinophil count was $<10\%$. According to these criteria, 14 sarcoidosis patients and nine IPF patients had a normal BAL cell differentiation. All 20 remaining patients with sarcoidosis had lymphocyte alveolitis without increased

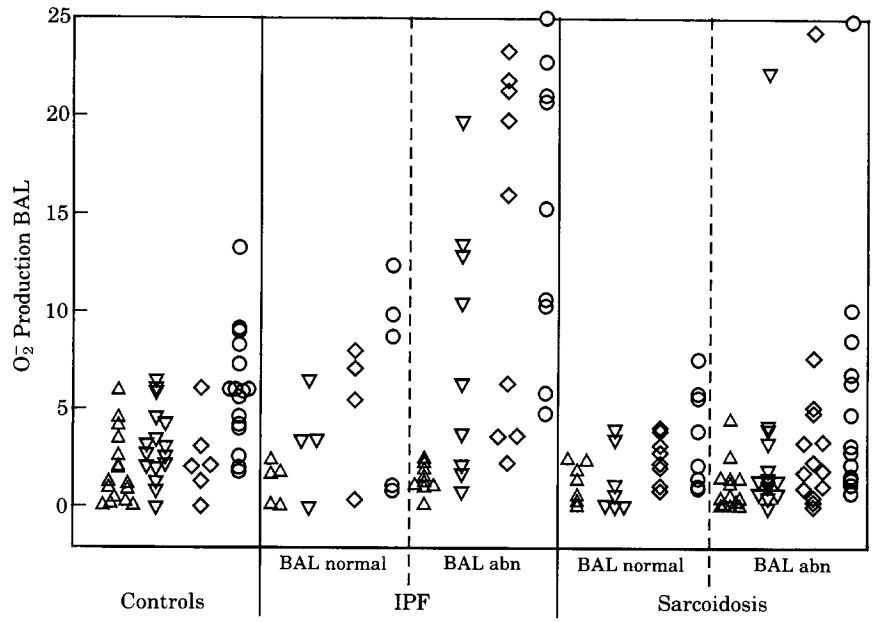

Fig. 1 O_2^- production by BAL cells in all subject groups, distinguished by BAL cell profile. O_2^- on *y*-axis expressed as nmol cytochrome *c* reduction per 15 min by 400 000 BAL cells. (△) Unstimulated O_2^- production; (▽) O_2^- production with PMA 5 ng ml^{-1}; (◇) O_2^- production with PMA 10 ng ml^{-1}; (○) O_2^- production with PMA 20 ng ml^{-1}. BAL abn: abnormal BAL cell differentiation (see text for criteria).

PMN percentages. In the 14 IPF patients with abnormal cell differentiations simultaneous increases in the percentage of lymphocytes and PMN were observed.

BAL lymphocyte phenotypes

Due to an insufficient number of cells recovered or a small percentage of lymphocytes, BAL lymphocyte phenotypes could not be determined in 16 controls and 24 patients. BAL lymphocytes were analysed in eight non-smoking controls. In the patient groups the ranges of the lymphocyte subsets were broader than in the healthy controls. BAL lymphocyte phenotypes showed characteristic changes for IPF and sarcoidosis (Table 1). In both diseases the proportion of activated lymphocytes, as measured by expression of HLA-Dr, was increased as compared to normals.

Sarcoidosis patients with lymphocyte alveolitis had a significantly increased percentage of CD4-positive cells ($57{\cdot}3 \pm 4{\cdot}1$ *vs.* $45{\cdot}0 \pm 2{\cdot}3$, $P<0{\cdot}01$), a decreased percentage of CD8-positive cells ($20{\cdot}9 \pm 2{\cdot}4$ *vs.* $32{\cdot}3 \pm 1{\cdot}7$, $P<0{\cdot}01$) and a higher CD4/CD8 ratio ($3{\cdot}68 \pm 0{\cdot}61$ *vs.* $1{\cdot}40 \pm 0{\cdot}02$, $P<0{\cdot}01$) in BAL than healthy controls. Sarcoidosis patients with normal BAL cell differentiation did not differ significantly from controls.

BAL oxygen radical production

Data from six controls, 14 IPF patients (five with normal BAL cell differentiation and nine with abnormal differentiation, data not shown) and 22 sarcoidosis patients (eight with normal BAL differentiation and 14 with lymphocyte alveolitis) were obtained. In addition to these data, ten healthy controls were included in whom only BAL cell differentiation and BAL O_2^- production were determined (see Fig. 1). When the BAL cell differentiation was normal, O_2^- production was similar in patients and healthy controls. As a whole, the group of patients with IPF produced significantly more O_2^- after stimulation with PMA than the other groups; PMA 5 ng ml^{-1}: $P<0{\cdot}05$ *vs.* sarcoidosis; PMA 10 and 20 ng ml^{-1}: $P<0{\cdot}025$ *vs.* controls, $P<0{\cdot}005$ *vs.* sarcoidosis, presumably as a result of the high proportions of neutrophils and eosinophils in these patients. However, no differences in O_2^- production were found between IPF patients with normal and abnormal BAL cell profiles.

Expressed per 400 000 AM, patients with sarcoid lymphocyte alveolitis produced significantly more O_2^- after stimulation with PMA, than sarcoidosis patients with normal BAL cell profiles (see Fig. 2). These results remained the same when only non-smokers were analysed.

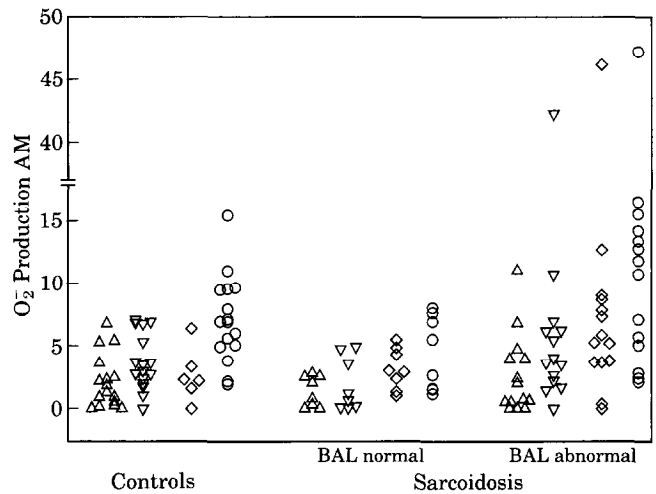

Fig. 2 O_2^- production by alveolar macrophages (AM) in healthy controls and patients with sarcoidosis, distinguished by BAL cell profile. O_2^- on *y*-axis expressed as nmol cyotchrome *c* reduction per 15 min by 400 000 AM (corrected for % of lymphocytes and polymorphonuclear cells). (△) Unstimulated O_2^- production; (▽) O_2^- production with PMA 5 ng ml^{-1}; (◇) O_2^- production with PMA 10 ng ml^{-1}; (○) O_2^- production with PMA 20 ng ml^{-1}. BAL abn: abnormal BAL cell differentiation (see text for criteria).

SYSTEMIC DISEASE MANIFESTATIONS

Blood lymphocyte phenotypes

Blood lymphocytes were analysed in eight non-smoking controls, 11 IPF patients and 23 sarcoidosis patients (data not shown). The percentage of T-lymphocytes was increased in both patient groups as compared to the healthy controls. This could not be attributed to an increase in one particular phenotype. Rather, a decreased percentage of CD8-positive cells in patients with sarcoidosis was observed ($P<0.05$). Percentages of B-cells, CD4-positive cells and CD4/CD8 ratios were similar in both patient groups and controls.

Blood PMN oxygen radical production

Oxygen radical (O_2^-) production of blood PMN was measured in nine healthy controls, 14 IPF and 26 sarcoidosis patients (data not shown). The O_2^- production of healthy controls was not significantly different from that of patients with IPF. Patients with sarcoidosis had higher spontaneous and stimulated O_2^- production rates in blood than the healthy controls, being significant for PMA 10 ng ml^{-1} (basal: 1.30 ± 0.25 *vs.* 0.97 ± 0.21, $P=0.08$; PMA 5 ng ml^{-1}: 12.39 ± 1.50 *vs.* 7.82 ± 0.90, $P=0.12$; PMA 10 ng ml^{-1}: 18.19 ± 1.59 *vs.* 10.54 ± 1.02, $P<0.05$).

RELATIONSHIPS BETWEEN BAL AND OTHER PARAMETERS OF DISEASE

Disease duration

Disease duration in patients with sarcoidosis showed a significant inverse correlation with the number of BAL cells per ml of BAL fluid ($r=-0.38$, $P<0.05$), the percentage and absolute number of lymphocytes in BAL ($r=-0.34$, $P<0.05$ and $r=-0.44$, $P<0.01$, respectively), as well as the number of CD4-positive cells in BAL ($r=-0.43$, $P<0.05$) and the CD4/CD8 ratio ($r=-0.48$, $P<0.025$). In IPF, disease duration did not correlate with BAL parameters.

Lung function

Patients with IPF had significantly lower lung volumes and diffusing capacity than patients with sarcoidosis (data not shown). In the latter group, pulmonary function, especially TLC, was directly related to BAL numbers of T-lymphocytes per ml ($r=0.54$, $P<0.025$), CD4-positive cells per ml ($r=0.57$, $P<0.01$) and B-cells per ml ($r=0.67$, $P<0.005$). By contrast, TLC was inversely correlated with O_2^- production, both PMA-stimulated [$r=-0.67$ ($P<0.002$) with 20 ng ml^{-1} PMA] and unstimulated ($r=-0.46$, $P=0.05$). Similar relationships with stimulated O_2^- production were seen for

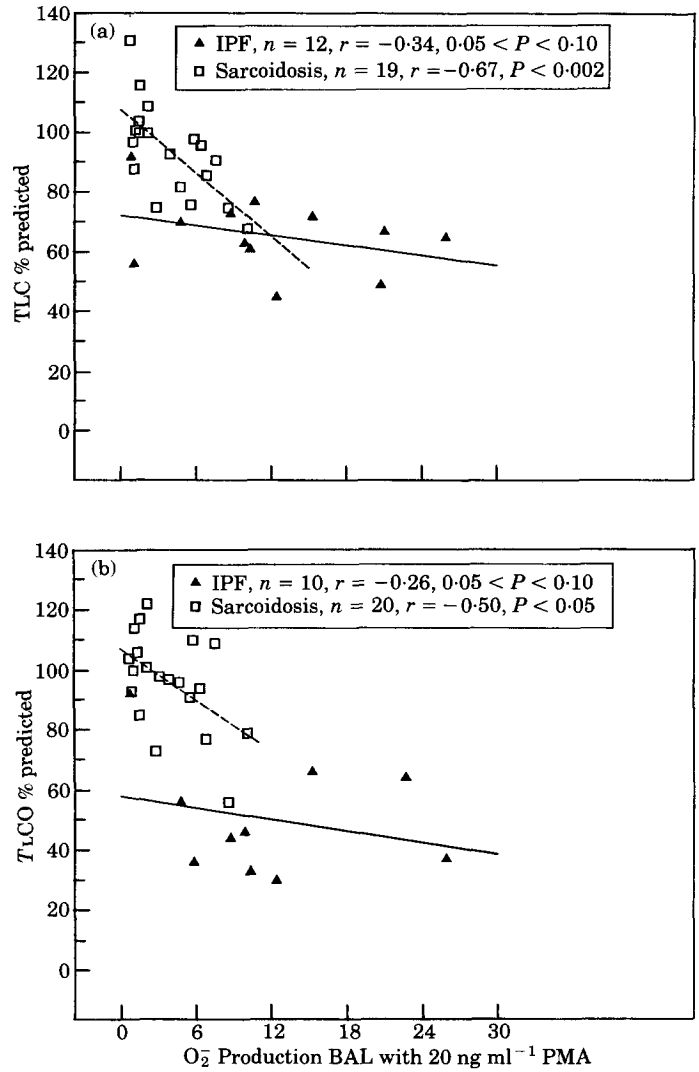

Fig. 3 Relationship between BAL O_2^- production and TLC (a) and *T*LCO (b) in patients with sarcoidosis and IPF. O_2^- expressed as nmol cytochrome *c* reduction in 15 min; solid regression lines: IPF; dashed regression lines: sarcoidosis.

*T*LCO: $r = -0{\cdot}50$ ($P<0{\cdot}05$) with 20 ng ml^{-1} PMA (Fig. 3). In patients with IPF, on the other hand, no clearly significant relationships could be found, although IVC, TLC and *T*LCO tended to correlate with PMA-stimulated O_2^- ($0{\cdot}05<P<0{\cdot}10$).

Clinical symptoms

Patients with extra-pulmonary sarcoidosis had a shorter disease duration ($P<0{\cdot}01$) than those with strictly pulmonary disease. Furthermore, patients with extra-pulmonary manifestations also had a higher percentage of CD4-positive cells ($P<0{\cdot}05$), a lower percentage of CD8-positive cells ($P<0{\cdot}025$) and accordingly a higher CD4/CD8 ratio ($P<0{\cdot}025$, Fig. 4). There were no differences in pulmonary function, blood lymphocyte phenotype or O_2^- production.

Chest roentgenogram

Chest X-rays of eight patients with sarcoidosis showed stage I abnormalities, seven had stage II and 17 had stage III. Lymphocyte alveolitis was associated with stage I and III, as opposed to normal BAL in patients with stage II X-rays. No differences in pulmonary function, O_2^- production by BAL cells or disease duration were observed. All IPF patients had interstitial abnormalities on their chest X-ray.

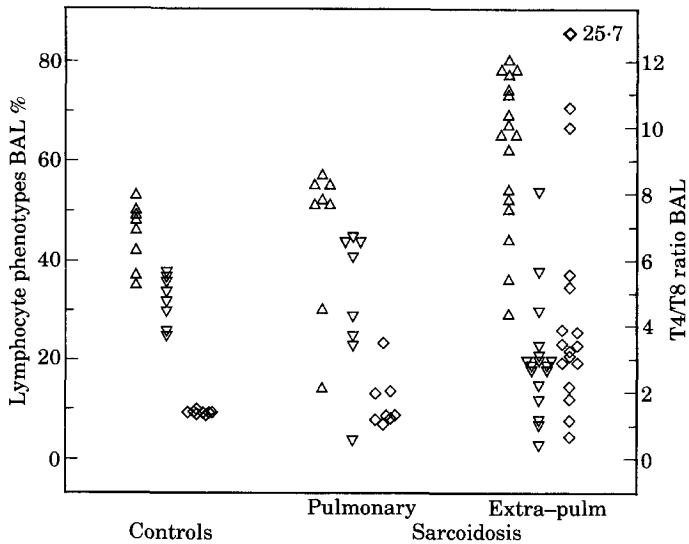

Fig. 4 Lymphocyte phenotypes in BAL of healthy controls and patients with sarcoidosis. Lymphocyte phenotypes expressed as percentages of the total number of lymphocytes. (△) CD4-positive cells; (▽) CD8-positive cells; (◇) CD4 to CD8 ratio (plotted against right *y*-axis); pulmonary: sarcoidosis restricted to the lungs; extra-pulm: pulmonary sarcoidosis with extra-pulmonary manifestations.

Angiotensin converting enzyme

Serum ACE levels in our sarcoidosis patients ranged from 5–89 U ml^{-1} (normal values <45 U ml^{-1}). We found a positive correlation between serum ACE levels and PMA-stimulated O_2^- production by BAL cells (PMA 10: $r=0{\cdot}54$, PMA 20: $r=0{\cdot}50$, $P<0{\cdot}025$ in both analyses).

Discussion

In the present study we investigated the relationship between clinical, roentgenological, lung functional and BAL-derived parameters of interstitial lung disease in patients with sarcoidosis and idiopathic pulmonary fibrosis (IPF) in order to reveal clues pointing to different pathogenetic mechanisms in these diseases. Indeed, we found that correlations between BAL and conventional clinical parameters were more notable in our patients with sarcoidosis than in those with IPF and suggest that this may reflect the fact that the latter group had more long-standing disease than the patients with sarcoidosis. In both diseases, lung lymphocytes showed increased expression of HLA-Dr, indicative of increased lymphocyte activation. Short disease duration in sarcoidosis was associated with more severe abnormalities of BAL parameters and with the presence of extra-pulmonary symptoms. Sarcoid lymphocyte alveolitis was associated with increased activation of alveolar macrophages as assessed by oxygen radical (O_2^-) production. In sarcoidosis high BAL O_2^- production was associated with more severe impairment of lung function, especially of TLC and *T*LCO. We also found that high serum ACE levels were associated with increased O_2^- production by BAL cells. In IPF, although O_2^- production by BAL cells was higher than in sarcoidosis and controls, there was no significant correlation with lung function.

Our data concerning cell composition and lymphocyte phenotypes in BAL fluid compare well with data found by others (1–3,13–15). Lymphocyte alveolitis is a common finding in sarcoidosis, whereas neutrophils and eosinophils usually dominate the alveolitis in IPF. The predominance of CD4 positive helper/inducer T-cells in sarcoidosis and of CD8 positive suppressor/cytotoxic T-cells in IPF is also well established. In addition, our measurements of HLA-Dr expression revealed that significant proportions of the lung lymphocytes were activated, in IPF as well as in sarcoidosis, but we did not distinguish between CD4 and CD8 positive cells in this respect. Intriguingly, the mechanisms of these differences remain unclear and our data do not allow us to elaborate on them.

In the case of sarcoidosis at least, disease duration is a critical factor in the analysis of BAL data. Ward *et al.* (22) found higher lymphocyte numbers and CD4/CD8 ratios in BAL in patients with shorter disease duration. Our findings confirm these relationships. In addition, we found that patients with

extrapulmonary symptoms, such as erythema nodosum and uveitis had more severe disruptions of lung lymphocyte phenotypes than patients with sarcoidosis restricted to the lungs. The presence of extrapulmonary symptoms was also associated with short diseases duration, which probably explains the relationships with lung lymphocyte phenotypes.

The presence of extrapulmonary manifestations was not reflected by, abnormalities of the lymphocyte phenotypes in blood. Apparently, the organs affected act immunologically independent.

Oxygen radicals, i.e. highly reactive oxygen metabolites produced by AM and PMN, have been incriminated in the pathogenesis of ILD by several authors (6,7,23,24) especially in IPF. Consistent with this, we have also found elevated O_2^- production by BAL cells in the patients with IPF in this study. In sarcoidosis, AM are the most important sources of O_2^- (5,25), and an upregulatory effect of T-lymphocytes has been proposed (8). Indeed, we found relationships between oxygen radical production, lymphocyte alveolitis and lung function compatible with this hypothesis, but only in sarcoidosis. The relationship between oxygen radical production and lung function impairment, however, largely depends on the temporal relationship between the release of O_2^- and the occurrence of lung function disturbances. Lung function reflects the cumulative effect of lung damage in the past and present, whereas O_2^- production is a parameter of current cell activation. Therefore, the relationships between these parameters should be assessed at an early stage in the disease, when previous episodes of increased O_2^- release do not mask the relationships. Thus, disease duration is a critically important factor in the interpretation of these results. Unfortunately, IPF has an insidious onset and the absence of early clinical signs, such as erythema nodosum in sarcoidosis, usually prevents an early diagnosis. This may explain why we did not find a relationship between BAL O_2^- production and lung function or between lymphocyte phenotypes and disease duration in IPF. The correlation of serum ACE levels with BAL O_2^- production in sarcoidosis may be seen as the simultaneous reaction of two markers of cellular activity. In addition, we found that BAL lymphocytes in sarcoidosis was associated with increased O_2^- production by BAL cells, suggesting that in active sarcoidosis lymphocytes may upregulate O_2^- production by AM.

These considerations imply that activation parameters in these diseases have to be interpreted with caution in clinical practice. Further studies of patients in a comparable stage of the disease and follow-up investigations will be necessary to define the significance of abnormalities at a given point in the course of the disease.

In conclusion, our findings show that lung lymphocyte phenotypes differ among patients with pulmonary and extrapulmonary sarcoidosis and that O_2^- production by BAL cells is upregulated in active pulmonary sarcoidosis. In addition, our findings stress the importance of disease duration in the analysis of BAL data and their relationship to the clinical disease state in sarcoidosis. In IPF, given its insidious onset, relationships between BAL parameters and lung function are difficult to interpret because disease duration is difficult to assess.

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